J. exp. Biol. (1982), 101, 35–45
With 3 figures
Printed in Great Britain

OXYGEN UPTAKE IN A SPONTANEOUSLY VENTILATING, BLOOD-PERFUSED TROUT PREPARATION

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(*Received* 3 *December* 1981 – *Accepted* 16 *April* 1982)

SUMMARY

1. A spontaneously ventilating, blood-perfused trout preparation was used to examine gas exchange across the gills.

2. The blood flow rate and input oxygen content to the branchial circulation were manipulated to assess the contributions of perfusion and diffusion limitations to oxygen transfer.

3. Increases in the flow rate ($\dot{Q}$), or the haematocrit (Hct) were positively correlated with increases in the oxygen uptake across the gills ($\dot{M}_{g,O_2}$).

4. Manipulation of pulse pressure or frequency of the pump, with no changes to $\dot{Q}$ had no effect on $\dot{M}_{g,O_2}$. Addition of adrenaline (1×10^{-6} M) to the blood also did not effect $\dot{M}_{g,O_2}$.

5. Calculations of cardiac output from the Fick principle always yielded values which were overestimates of the actual cardiac output ($\dot{Q}$) set by the mechanical pump.

6. The difference between the measured oxygen uptake by the fish from the water ($\dot{V}_{g,O_2}$) and the amount of oxygen transferred to the blood across the gills ($\dot{M}_{g,O_2}$) was a reflection of gill tissue metabolism.

7. It is concluded that trout gills, like mammalian lungs, are primarily perfusion limited for oxygen uptake under resting normoxic conditions, but decreases in diffusion limitations come into play under stress conditions, such as environmental hypoxia or exercise.

INTRODUCTION

Oxygen uptake in fish, and the transfer of oxygen across fish gills have been the subjects of numerous studies (Fisher, Coburn & Forster, 1969; Randall, 1970; Cameron & Davis, 1970; Brett, 1972; Jones & Randall, 1978). Fisher *et al.* (1969) have shown that carbon monoxide transfer across fish gills is diffusion limited and calculated that the gills also must be diffusion limited for oxygen. Certainly there is a large boundary layer of water and a thick gill epithelium separating water flowing over the gills from blood perfusing the gills. In addition, there are persistent and sometimes large P_{O_2} differences between blood and water across the gill, indicating

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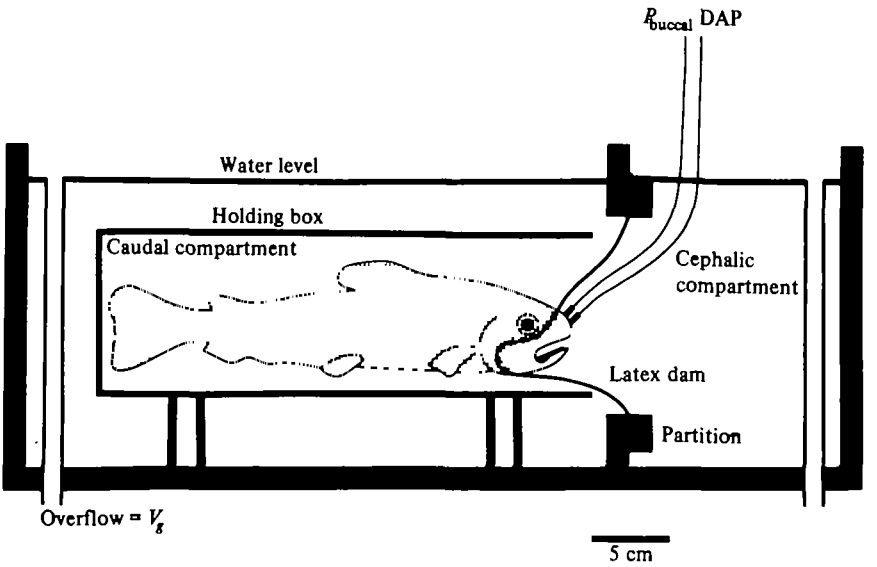

Fig. 1. Diagrammatic representation of the modified van Dam apparatus used in the present experiments. The Latex dam separates inspiratory water from expired water. Therefore, the overflow in the caudal compartment of the holding chamber is that volume of water which the fish ventilates over its gills (V_g). A supply of aerated or hypoxic water, far in excess of ventilation volume, is maintained to the cephalic compartment.

that arterial blood does not equilibrate with inflowing water. These results also have led to assumptions that fish gills are primarily diffusion limited for oxygen (Randall, 1970; Piiper & Scheid, 1977; Jones & Randall, 1978; Hughes, 1980).

Dorsal aortic blood, however, remains at least 95% saturated with oxygen over a wide range of conditions, including exercise. During exercise, blood flow through and water flow over the gills are increased, as is the arterial–venous oxygen content difference, resulting in a marked rise in oxygen uptake (Kiceniuk & Jones, 1977). Exercise also is associated with an increase in the gill oxygen 'transfer factor' (Randall, Holeton & Stevens, 1967), but whether this is due to changes in perfusion, or conditions for diffusion, or both, is not clear.

The blood-perfused trout preparation previously described by Davie, Daxboeck, Perry & Randall (1982) affords an opportunity to study the problem of perfusion versus diffusion limitations for O_2 transfer because, unlike in the intact animal, blood flow rate and input blood oxygen content can be altered and manipulated independently. In this way the relative importance of changes, known to be associated with alterations in oxygen uptake across the gills of intact rainbow trout (*Salmo gairdneri*), can be assessed.

MATERIALS AND METHODS

The procedures for setting up blood-perfused fish (*Salmo gairdneri*), methods of sampling, and analysis have been described in detail in the preceding paper (Davie *et al.* 1982). In the present study, fish were subjected to changes in blood perfusion rate and to changes in haematocrit, in order to investigate their effect on oxygen transfer. Input

Table 1. *The effects of changes in input blood flow on blood oxygen levels, oxygen uptake by the blood ($\dot{M}_{g,O_2}$) and the respiratory gas exchange ratios across the gills (RE_g) and the systemic circulation (RQ_s) of spontaneously ventilating, blood-perfused trout*

(N = 7 fish, weight 292·7 ± 14·3 g.)

	Input blood flow ($\dot{Q}$)		
	Low	Normal	High
$\dot{Q}$(ml.min^{-1}.fish^{-1})	2·76 ± 0·24	4·80 ± 0·20	7·20 ± 0·04
Input blood haematocrit (%)	9·34 ± 0·5	9·6 ± 0·4	9·6 ± 0·5
Input blood P_{O_2} (mmHg)	41·3 ± 3·0	28·7 ± 1·9	21·6 ± 1·6
Input blood C_{O_2} (mM)	1·06 ± 0·14	0·76 ± 0·05	0·74 ± 0·06
Dorsal aorta haematocrit (%)	7·8 ± 0·5	8·5 ± 0·5	8·5 ± 0·6
DA blood P_{O_2} (mmHg)	104·9 ± 2·4	103·6 ± 4·7	109·5 ± 3·1
DA blood C_{O_2} (mM)	1·46 ± 0·15	1·34 ± 0·09	1·42 ± 0·13
$\dot{M}_{g,O_2}$ (μmol.min^{-1}.100 g^{-1})	0·46 ± 0·07	1·17 ± 0·08	1·56 ± 0·10
RE_g	2·82 ± 0·87	1·65 ± 0·34	1·34 ± 0·33
RQ_s	1·23 ± 0·19	1·06 ± 0·17	0·82 ± 0·08

blood flow to the branchial circulation from the cardiac pump was adjusted initially to give a dorsal aortic blood pressure of 40 cm H_2O. Changes then were made in the pattern and volume of blood perfusion into the fish with no interruption of blood flow. Following a 5 min stabilization period, various parameters were measured under the new set of conditions. Fish were subjected to the following changes in no particular sequence:

(i) Input flow ($\dot{Q}$) was increased by 1·5 times the initial blood flow by elevating stroke volume, keeping frequency of the pump at 40.min^{-1}. No adjustment was made to input pulse pressure.

(ii) Input flow was decreased by 0·5 times the initial blood flow by decreasing stroke volume with no change in pump frequency.

(iii) Input pulse pressure was raised by doubling stroke volume and halving pump frequency, to keep flow constant at the initial level.

(iv) Input haematocrit was doubled by adding red blood cells to the tonometer, while input flow remained at the initial level.

(v) Input haematocrit was reduced to approximately 4%, while no other changes were made.

In all experiments, fish irrigated their gills with aerated water supplied from a flow-through reservoir maintained at 7 °C.

In a second series of experiments, water flow over the gills, oxygen loss from water to fish ($\dot{V}_{g,O_2}$), and a variety of blood parameters were measured. The fish used in these experiments (N = 4; 340·1 ± 13·2 g) were prepared as before, but in addition, the thumb cut from a thin Latex glove was used as a dam to separate inspired and expired water (Fig. 1, Van Dam apparatus) so that ventilatory flow ($\dot{V}_g$) could be measured (see Cameron & Davis, 1970; Randall & Jones, 1973). This procedure also permitted the measurement of inspiratory (P_{I,O_2}) and mixed expired (P_{E,O_2}) water oxygen tensions. The inflowing water to this apparatus (Fig. 1) was passed through a gas exchange column in order to control inspiratory water P_{O_2}. P_{I,O_2} was maintained at

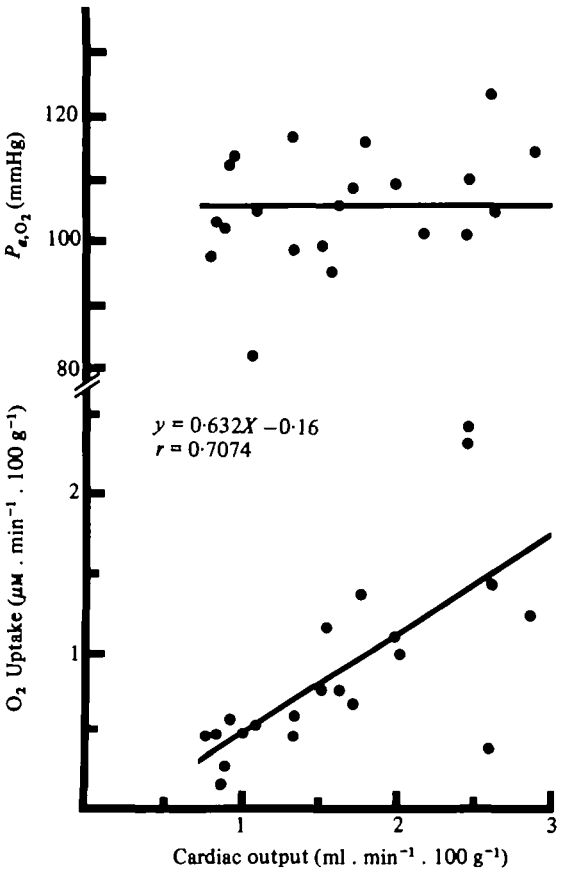

Fig. 2. The effects of changes in cardiac output on oxygen uptake and P_{a,O_2} across the gills of spontaneously ventilating, blood-perfused trout.

either normoxic levels (151·4 ± 5·4 mmHg) or hypoxic levels (81·0 ± 5·4 mmHg). In both cases, input flow rate ($\dot{Q}$) was kept constant, but during hypoxia, pump frequency was halved and stroke volume doubled. This manipulation was intended to simulate the bradycardia observed in intact fish in response to environmental hypoxia.

All data are presented as means ± standard error of the mean (S.E.M.). Results were analysed using the Student's t test and 10 or 5% was taken as the fiducial limit of significance, unless otherwise stated.

RESULTS

The effects of changes in input blood flow ($\dot{Q}$) on the cardiorespiratory system of spontaneously ventilating, blood-perfused trout preparations are presented in Table 1. Increasing input blood flow had no effect on either dorsal aortic blood P_{O_2} or oxygen content, but resulted in an increase in oxygen uptake by the blood across the gills ($\dot{M}_{g,O_2}$). There was a significant positive correlation between the rate of blood flow

and oxygen uptake across the gills (Fig. 2). The linear relationship between blood flow and oxygen uptake is represented by the following equation:

$$\dot{M}_{g,O_2}(\mu\text{mol.min}^{-1}.100\ \text{g}^{-1}) = 0{\cdot}63\ \dot{Q}(\text{ml.min}^{-1}.100\ \text{g}^{-1}) - 0{\cdot}16 \quad (1)$$
$$(P \leqslant 0{\cdot}01;\ n = 21 \text{ observations}).$$

Increasing flow reduced the P_{O_2} and oxygen content of input blood below resting levels. However, the P_{O_2} and oxygen content of venous blood returning to the tonometer was unchanged from resting values by increases in blood flow. The blood turned over more rapidly in the tonometer at higher blood flows and therefore had less time to reach equilibrium with the inflowing gas mixture. Thus we observed an inverse relationship between flow rate and input blood P_{O_2} and C_{O_2}. The gaseous (tonometer) P_{O_2} was higher (approx. 60 mmHg) than that of returning mixed venous blood (12–14 mmHg) and even at the lowest flow, blood in the tonometer reached a P_{O_2} of only $41{\cdot}3 \pm 3{\cdot}0$ mmHg.

The P_{O_2} and oxygen content of blood in the dorsal aorta was similar at all flow rates. P_{O_2} was reduced by 87%, and the oxygen content by 76% of the input value as blood flowed through the systemic circuit, regardless of blood flow rate. Thus oxygen uptake by the tissues was directly proportional to blood flow.

Ventilation volume was not measured in the above experiments and therefore we cannot state whether it was affected by changes in $\dot{Q}$. However, we recorded no changes in either the ventilation rate or the amplitude of the buccal pressure when $\dot{Q}$ was changed, and therefore we are assuming that ventilation volume also was unaffected.

The effects of increasing input blood haematocrit on oxygen transfer in the blood-perfused trout preparations are shown in Table 2. There were no significant changes in dorsal aortic blood P_{O_2}. At these O_2 tensions blood leaving the gills will be saturated with oxygen and the increasing oxygen content simply is a reflexion of the increasing haemoglobin content associated with elevated haematocrit.

Oxygen uptake across the gills into the blood was positively correlated with haematocrit (Fig. 3), the relationship being described by the following equation:

$$\dot{M}_{g,O_2}(\mu\text{mol.min}^{-1}.100\ \text{g}^{-1}) = 0{\cdot}13\ \text{Hct}\,(\%) - 0{\cdot}14 \quad (2)$$
$$(P \leqslant 0{\cdot}01;\ n = 19 \text{ observations}).$$

The P_{O_2} of blood returning to the tonometer from the fish was unaffected by haematocrit, but the P_{O_2} of the input blood decreased with the increasing haematocrit. Although blood residence time in the tonometer did not vary, the oxygen load did, with haematocrit, and therefore the blood P_{O_2} in the tonometer was further from equilibrium with the inflowing gas mixture when haematocrit was elevated. This was a result solely due to the conditions for equilibration in the tonometer.

The gas exchange ratios across the gills ($\dot{M}_{g,CO_2}/\dot{M}_{g,O_2} = RE_g$) are very high but decrease with increasing blood flow (Table 1) and haematocrit (Table 2). These unusual values are a reflexion of the variable oxygen content, but more stable carbon dioxide content, of input blood (see Davie *et al.* 1982 for detailed discussion). The gas exchange ratio across the systemic circuit (RQ_s) however, is more representative of the tissue respiratory quotient and ranges from 0·67 to 1·23 (Tables 1 and 2). The average RQ_s for all conditions was 0·92.

Table 2. *The effects of changing haematocrit on oxygen transfer in spontaneously ventilating, blood-perfused trout*

(N = 6 fish; 320·2 ± 17·2 g. DA, dorsal aortic; $\dot{M}_g$, O_2 oxygen uptake by blood flowing through the gills; RE_g, gas exchange ratio in the gills; RQ_s, gas exchange ratio in the systemic circulation.)

	Input blood haematocrit		
	Low	Normal	High
Input blood haematocrit (%)	4·3 ±0·4	11·3 ±0·5	20·2 ±1·6
Input blood P_{O_2} (mmHg)	39·4 ±4·2	20·3 ±2·5	16·3 ±2·6
Input blood O_2 content (mM)	0·59±0·03	0·95±0·20	1·37±0·26
DA blood haematocrit (%)	3·9 ±0·5	9·3 ±0·5	16·5 ± 0·5
DA blood P_{O_2} (mmHg)	108·7 ±5·8	95·3 ±6·6	91·6 ±13·2
DA blood O_2 content (mM)	0·87±0·12	1·77±0·20	2·88±0·40
$\dot{M}_{g,O_2}$ (μmol.min^{-1}.100 g^{-1})	0·46±0·18	1·34±0·19	2·45±0·40
RE_g	2·9 ±0·4	2·0 ±0·1	1·7 ±0·3
RQ_s	0·85±0·44	0·67±0·11	0·87±0·16
Input blood flow, $\dot{Q}$ (ml.min^{-1}.fish^{-1})	5·20±0·04	5·20±0·04	5·20±0·04

Table 3. *Summary of the cardio-respiratory variables from normal and hypoxic spontaneously ventilating, blood-perfused trout in a modified van Dam apparatus*

(N = 4 fish; 340·1 ± 13·2 g.)

	Normoxia (A)	Hypoxia (B)	Change (%) $\frac{B-A}{A}\times 100$
P_{I,O_2} (mmHg)	151·4±0·8	81·0±5·4	−46
P_{E,O_2} (mmHg)	120·0±3·6	68·7±5·4	−43
$\dot{V}_g$ (ml.min^{-1})	57·4±6·42	96·6±13·3	+68
$\dot{V}_g:\dot{Q}$	9·47±0·82	16·2±1·7	+71
$\dot{M}_{g,O_2}$ (μmol.min^{-1}.100 g^{-1})	1·083±0·103	0·570±0·04	−47
Input blood P_{O_2} (mmHg)	22·7±0·86	23·3±1·45	+3
Input blood O_2 content (mM)	0·69±0·06	0·73±0·05	+5
DA blood P_{O_2} (mmHg)	95·9±3·87	51·5±5·1	−46
DA blood O_2 content (mM)	1·26±0·07	1·101±0·08	−13
Venous return O_2 content (mM)	0·315±0·04	0·249±0·03	−21
Input pressure (cm H_2O)	52·6±1·1	54·7±2·6	+4
DA pressure (cm H_2O)	35·4±2·4	31·9±1·7	−10
R_g (cm H_2O.ml^{-1}.min.100 g^{-1})	8·80±0·99	12·25±1·10	+39
R_s (cm H_2O.ml^{-1}.min.100 g^{-1})	17·58±1·33	17·11±1·05	−3
n (observations)	10	8	

Manipulations of pump stroke volume, frequency and input pulse pressure had no significant effect on oxygen uptake across the gills when perfusion rate ($\dot{Q}$) was held constant. The addition of adrenaline to the blood (1×10^{-6} M) also caused no change in $\dot{M}_{g,O_2}$; pre-adrenaline exposure $\dot{M}_{g,O_2}$ was 1·10±0·23 μmol.min^{-1}.100 g^{-1}, whereas following the addition of adrenaline to the blood, $\dot{M}_{g,O_2}$ was 1·30± 0·25 μmol.min^{-1}.100 g^{-1}. Other effects of changes in input pressure and flows, and the addition of adrenaline to the blood on the cardiovascular system of this blood-perfused preparation have been described in the preceding paper (Davie *et al.* 1982). Again, in all of the above experiments ventilation rate and buccal pressures remained unchanged from normal.

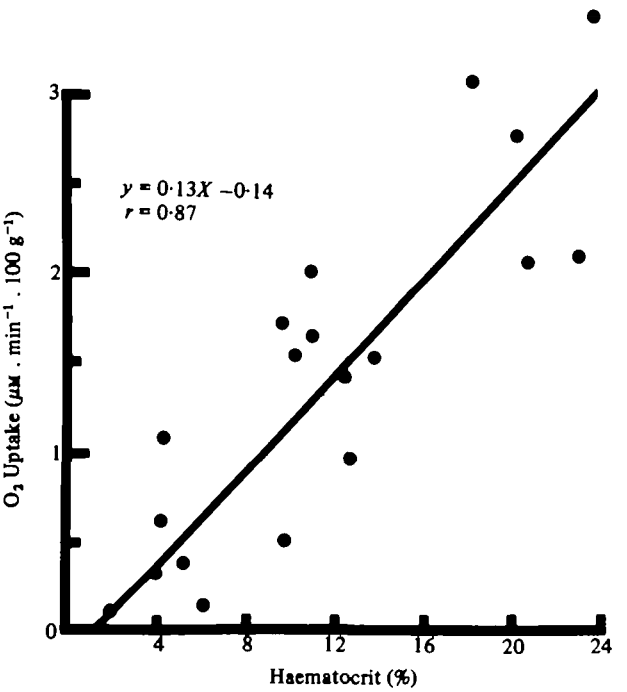

Fig. 3. The effect of variable input haematocrit on oxygen uptake across the gills of spontaneously ventilating, blood-perfused trout.

Direct measurement of ventilation volume

Placing fish in a van Dam apparatus had no effect on the blood variables measured in the blood-perfused fish during normoxia (Table 3, cf. data of Davie *et al.* 1982). Ventilation volume was 57·4 ml . min^{-1}, giving a $\dot{V}_g : \dot{Q}$ ratio of about 10, a value characteristic of this fish. During mild hypoxia, $\dot{V}_g$ increased as did the $\dot{V}_g : \dot{Q}$ ratio. Unlike the intact fish, oxygen uptake by the blood decreased during hypoxia, and is largely a reflexion of the stable level of input blood oxygen content associated with a fall in dorsal aortic blood oxygen content. In intact fish, venous oxygen content shows a marked reduction during hypoxia and oxygen uptake is maintained at normoxic levels (Holeton & Randall, 1967). Oxygen extraction by the tissues in the systemic circulation decreased slightly during hypoxia as did the oxygen content of input (DA) blood to the systemic circulation. The result was that the percent oxygen extraction by systemic tissues was the same during normoxia and hypoxia (77·4 ± 2·0% of dorsal aortic blood oxygen content). Simulated bradycardia during hypoxia did not affect either oxygen removal from the water or uptake by the blood. Nor were there any significant changes in any of the blood gas levels as a result of the change from high rate/low stroke volume to low rate/high stroke volume during hypoxia. The gill resistance to blood flow (R_g) increased significantly during hypoxia, but this increase was not so apparent with simulated bradycardia, which caused a non-significant decrease in hypoxic R_g values. The systemic resistance (R_s) was unchanged during hypoxia, with or without bradycardia.

Table 4. *The difference between oxygen uptake by the blood as it perfuses the gills ($\dot{M}_{g,O_2}$) and the amount leaving the water as it flows over the gills ($\dot{V}_{g,O_2}$). This difference is due, presumably, to oxygen utilization by the gill tissues*

	Normoxia	Hypoxia
$\dot{M}_{g,O_2}$ (μmol.min^{-1}.100 g^{-1})	1·083 ± 0·103	0·570 ± 0·04
$\dot{V}_{g,O_2}$ (μmol.min^{-1}.100 g^{-1})	1·653 ± 0·229	1·101 ± 0·07
Oxygen uptake by gill tissue (μmol.min^{-1}.100 g^{-1})	0·726 ± 0·184	0·532 ± 0·09
$\dot{Q}$ (ml.min^{-1}.Kg^{-1}) (actual)	16·81 ± 0·48	16·85 ± 0·85
$\dot{Q}$ (ml.min^{-1}.Kg^{-1}) (calculated by Fick principle)	25·12 ± 2·18	29·68 ± 3·20
n (observations)	9	7

It is possible to calculate the amount of oxygen removed from the water as it passes over the gills ($\dot{V}_{g,O_2}$) and to compare this with the amount of oxygen which enters the blood, calculated from flow and venous arterial oxygen content. The amount of oxygen removed from the water always exceeded the amount that entered the blood; the difference presumably being utilized by gill tissues (Table 4). In fact, the oxygen utilized by the gill tissue represented a median 27% (range 19·2–74·6%) of the total oxygen removed from the water as it passed over the gills. The oxygen utilization by gill tissues decreased slightly in hypoxia (Table 4).

DISCUSSION

The data presented in this study show that the gills of blood-perfused rainbow trout, like mammalian lungs, appear to be primarily perfusion limited for oxygen uptake under normoxic conditions. These data are probably applicable to intact fish because blood flow rates and oxygen uptake rates, as well as a variety of other parameters in the blood-perfused preparation are similar to those observed in intact trout (see Davie *et al.* 1982). One method the fish may use to increase oxygen uptake is to increase blood flow through the gills. It should be remembered that while $\dot{Q}$ was increased approximately 2-fold from rest, the fish was not capable of actually performing exercise in our experiments. Although this increase in $\dot{Q}$ can easily be accomplished by trout during swimming exercise (Kiceniuk & Jones, 1977), there also is a concomitant cardiac output redistribution in the systemic circulation (C. Daxboeck, D. J. Randall & D. R. Jones, in preparation) to accommodate the increased metabolic demand, a situation not simulated in the present experiments. Alternatively, the capacity of the blood to take up oxygen can be elevated, either by reducing venous blood oxygen content, or by increasing the blood haemoglobin content, for example by raising haematocrit. Such adjustments have been observed for fish *in vivo* (see Jones & Randall, 1978), and also were found to increase oxygen transfer across the gills in our preparations.

In the past it has been concluded (Jones & Randall, 1978) that the gills of fish are primarily diffusion limited rather than perfusion limited for two main reasons. First, post-branchial arterial blood is far from equilibrium with the P_{O_2} of inspiratory water. Second, Fisher *et al.* (1969) showed that the catfish gill is diffusion limited for carbon monoxide (i.e. the rate of carbon monoxide transfer is proportional to the difference in

D_{O} across the gills), and calculated that the gill also would be diffusion limited for oxygen. Carbon monoxide cannot be used by tissues for any metabolic purpose. However, the large P_{O_2} differences between water and blood across the gills can be accounted for qualitatively, by not only the diffusion barrier of the gill epithelium itself, and water boundary layer, but also by the oxygen utilization of the gill tissue. If, for instance, the mean P_{O_2} difference across the gills is 45 mmHg, then at least 27% of this difference can be accounted for by gill tissue oxygen utilization, thus reducing the apparent gradient for oxygen between blood and water to around 30 mmHg P_{O_2}. The gill epithelium is 5 μm thick and, given the thickness of the pillar cells and blood space, then using diffusion coefficients for oxygen in frog tissue (Randall, 1970) and resting oxygen uptake rates for trout, one might expect a P_{O_2} difference of 15–20 mmHg across the fish gill. The remainder of the P_{O_2} difference across the gill probably can be accounted for by the boundary layer of water at the gill/water interface (Piiper & Scheid, 1977). It should be pointed out that, under normal circumstances, O_2 must pass from the water into the epithelium and then into the blood. Therefore the epithelium P_{O_2} must be higher than the blood P_{O_2}, or else no oxygen could enter the blood, and thus all O_2 for epithelial metabolism probably is supplied from the water.

Conditions for diffusion in the gills do not change with hypoxia and exercise (Randall *et al.* 1967; Jones & Randall, 1978). For instance, lamellar recruitment probably not only increases blood residence time in the gills but also increases gill diffusing capacity. Lamellar recruitment is facilitated by increases in ventral aortic pulse pressure and increases in heart stroke volume (Farrell, Daxboeck & Randall, 1979; Farrell, Sobin, Randall & Crosby, 1980; Davie & Daxboeck, 1982), but these changes do not alter oxygen uptake in the blood-perfused preparation under normoxic resting conditions. In addition, increased circulating catecholamines are thought to increase gill oxygen diffusion coefficients, thus augmenting oxygen transfer (Peyraud-Waitzenegger, 1979). Elevating the adrenaline levels in the blood of our preparations does not raise oxygen uptake. However, with the cardiac output used in these experiments, the blood was near maximal O_2 carrying capacity. Under these conditions, changes in permeability or blood flow path caused by adrenaline would not be expected to increase oxygen uptake appreciably at constant $\dot{Q}$. We conclude that, in our normoxic and resting blood-perfused fish, oxygen transfer is not diffusion limited, and therefore these changes would have no effect. In the intact animal, however, especially during heavy exercise, when O_2 transfer is maximal and cardiac output is higher than normal, and during extreme environmental hypoxia, when P_{O_2} gradients are minimal, then increased gill diffusing capacity probably is of great importance in maintaining adequate rates of oxygen transfer. Thus, changes in diffusing capacity which may allow the maintenance of oxygen transfer in extreme hypoxia led Fisher *et al.* (1969) to conclude that catfish gills are diffusion limited. From our results it would appear, however, that in normoxia and moderate environmental hypoxia the rate of oxygen transfer measured at the gills is primarily perfusion limited. This conclusion is substantiated further by the observation that branchial CO_2 excretion in blood-perfused preparations also is related directly to the rate of perfusion (Daxboeck, 1981; see also Perry *et al.* 1982, for a detailed discussion of CO_2 excretion in fish). Changes

in blood distribution within the gills (i.e. lamellar recruitment), or increases in g[illegible] oxygen permeability due to elevated circulating catecholamine levels however are of physiological significance to oxygen transfer under extreme conditions.

Oxygen uptake by gill tissues

Our data indicate that a median 27% of the oxygen taken up by the resting fish is utilized by the gill tissue. Gill tissue constitutes 3·9% of the total body weight in trout (Stevens, 1968) and therefore our calculated gill tissue oxygen metabolism is 18·6 μmol.min^{-1}.100 g^{-1} of tissue. This value is similar to that recorded by Hochachka (unpublished observations) for a variety of Amazonian fish gills *in vitro*. In our experiments we measured the difference between the amount of oxygen removed from water and the amount entering the blood, the difference being the oxygen utilized by tissues in the buccal and opercular cavities and gills. The general body surface also utilizes oxygen from the water (Kirsch & Nonnotte, 1977), so the proportion of oxygen entering the blood when measuring total oxygen uptake by the fish will be even less than recorded here. Many investigators have calculated cardiac output, using the Fick principle, from measurements of oxygen removed from the water, and pre- and post-gill blood oxygen contents (see Jones & Randall, 1978). The assumption inherent in the use of the Fick calculation is that all of the oxygen removed from water enters the blood. This is clearly not the case for rainbow trout, and therefore cardiac output calculations are overestimates of the actual value. Hughes *et al* (1981) have made similar measurements on eels and found that the measured $\dot{Q}$ exceeded that obtained from calculations using the Fick principle by 37·7 ± 11·0%. This is the complete reverse of our observations, where Fick calculations yield an overestimate of the measured blood flow. Hughes *et al.* (1981) suggested that the difference in measured and calculated $\dot{Q}$ was due to the large gill venous by-pass in eels, so that not all the blood entering the gills is involved in oxygen uptake. This is not the case in trout where nearly all the blood must pass through the secondary lamellae and be oxygenated (Cameron, 1974). Thus, use of the Fick principle in estimating the cardiac output in fish is full of difficulties. Gill venous by-pass systems lead to underestimates whereas oxygen utilization from water by gill and skin tissues can lead to overestimates. Unless these two factors are quantified, the estimates of cardiac output using the Fick principle have little value.

This work was supported through N.S.E.R.C. grants to D.J.R. P.S. Davie was the recipient of a Killam Postdoctoral Fellowship.

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